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TECHNICAL REPORTS

Wetlands and Aquatic Processes

Impacts of a large invasive mammal on water quality in riparian ecosystems

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Abstract

Wild pigs (*Sus scrofa*) are a highly invasive species in many regions of the world and can act as ecosystem engineers in areas where they are established. In riparian ecosystems, wild pigs may affect water quality parameters and introduce fecal bacteria, although previous studies have reported conflicting results. We propose four conditions that we believe are needed for an accurate assessment of wild pig impacts on water quality and address each one in our study. Water samples were collected between May 2018 and June 2019 in riparian watersheds on a privately owned property in Alabama that was densely populated by wild pigs (treatment) and in watersheds at a nearby national forest without an established population. Samples were analyzed for concentrations of water quality parameters, such as anions and cations, dissolved oxygen, total suspended solids, N, dissolved organic C, and *Escherichia coli* and other fecal coliforms. An additional 38 samples were analyzed using quantitative polymerase chain reaction for swine fecal bacteroidetes. At treatment watersheds, specific conductivity and concentrations of organic N and C, SO_4^{2-} , and Ca^{2+} were between 2 and 11 times that of reference watersheds. *Escherichia coli* values at treatment watersheds were 40 times reference watershed values. DNA from swine fecal bacteroidetes was detected in 70% of treatment samples and 0% of reference samples. Wild pigs are a threat to water quality in riparian areas, and our results indicate that it may be important to control populations upstream of major drinking water sources and recreational areas.

1 | INTRODUCTION

As global development continues to bring urban and rural landscapes closer together, watershed health and security are increasingly threatened by changes in land use and the resulting environmental conditions. Urban development,

agricultural activities, and degradation and invasion of natural landscapes all influence local watershed health and can affect the quantity and quality of water available (Baldwin & Batzer, 2012). Riparian and wetland forest ecosystems play a vital role as the “kidneys” of a watershed because they filter pollutants and sediment from the aquatic system through biological, chemical, and physical means (Jolley et al., 2010). Removal of dissolved chemicals and sediment improves water quality, and nutrient uptake ensures adequate nutrient cycling through the terrestrial–aquatic interface. Riparian

Abbreviations: cfu, colony-forming unit; DO, dissolved oxygen; DOC, dissolved organic carbon; FC, fecal coliforms; MST, microbial source tracking; TN, total nitrogen; TSS, total suspended solids.

areas provide important ecological services, such as habitat and resources for plant and animal communities and surface water storage. Additionally, riparian areas support commercial industries (e.g., agriculture and livestock production) and recreational hunting and fishing activities.

Improper sanitation management, stormwater runoff from urban and residential areas, poor land-use practices, and agricultural runoff are some potential causes of degraded water quality in local watersheds. However, the presence of livestock and wildlife in a watershed may reduce water quality as well. Livestock may contaminate stream water through direct contact or from runoff contaminated with feces and urine (Davies-Colley et al., 2004; Line et al., 2000), and subsequent use of contaminated water has been linked to disease outbreaks in humans (Ackers et al., 1998; Jay et al., 2007; Lindqvist et al., 2008). Wildlife species have also been documented as a source of waterborne fecal bacteria pollution. *Escherichia coli* and enterococci from gull (*Larus* sp.) feces has been detected at beaches in the Great Lakes in Illinois and Michigan (Fogarty et al., 2003), and *E. coli* from white-tailed deer (*Odocoileus virginianus*) and Canada geese (*Branta canadensis*) has been found in watersheds of the Finger Lakes in New York (Somarelli et al., 2007).

Direct impacts by wildlife on other water quality parameters are less documented, but indirect impacts of wildlife feeding or nesting behaviors can be substantial. For example, grazing by Canada geese can reduce the abundance of wetland vegetation, thereby limiting the ability of a watershed to filter and retain sediment and nutrients (Baldwin & Pendleton, 2003). As ecosystem engineers, beavers (*Castor canadensis*) alter the vegetative structure, biogeochemistry, geomorphology, and hydrology of wetlands by felling trees and building dams (Johnston, 2012; National Research Council, 2002). For example, beaver impoundments can elevate the water table by controlling stormflow (Wigley & Lancia, 1998), reduce water velocity and streambank erosion (Maret et al., 1987), increase NH_4^+ concentrations in sediment (Naiman et al., 1994), and increase aquatic microbial activity (Songsteralpin & Klotz, 1995).

Wild pigs (*Sus scrofa*) are quickly gaining a reputation as a threat to wetland and riparian ecosystems. Invasive to North America and many other parts of the world, wild pigs occur at high densities throughout much of the southeastern United States and frequently use wetlands and riparian forests for their habitat requirements (Lewis et al., 2019; Mayer et al., 2020). Wild pigs dig and overturn soil in search of food and create wallows for thermoregulation and ecto-parasite removal (Bracke, 2011; Gray et al., 2020; Howe & Bratton, 1976). This rooting behavior can have serious consequences on the physical structure of ecosystems by causing increased erosion, destroying vegetative communities (Henry & Conley, 1972; Ralph & Maxwell, 1984; Wood & Roark, 1980), and introducing pathogens, parasites, and invasive species

Core Ideas

- Wild pigs are a threat to water quality in riparian areas.
- Wild pigs introduced fecal material and bacteria to streams.
- Watersheds with wild pigs had elevated DOC and TN compared with reference watersheds.
- *E. coli* values in treatment watersheds were 40 times those of reference watersheds.
- We propose four guidelines to assess the impacts of wild pigs on water quality.

(Chalkowski et al., 2018; Cushman et al., 2004; Seward et al., 2004). As a result, wild pigs have significant potential to alter the functionality of wetland and riparian ecosystems, and their impacts are of great concern because riparian ecosystems provide essential ecological services and are sensitive to disturbance (King, Battaglia et al., 2012; National Research Council, 2002).

Few studies have examined the impacts of wild pig disturbance on water quality and fecal bacteria in riparian areas, and those that have either did not find significant impacts or their results conflicted with other literature (e.g., Beasley et al., 2018). These inconsistencies have generally been due to variability in experimental design, environmental conditions, land cover and use, density of wild pigs, and parameters selected for measurement. Doupé et al. (2010) conducted a study in northeastern Australia during the dry season with small ephemeral lagoons and concluded that the ability of wild pigs to access lagoons affected some water quality parameters. Lagoons with wild pigs had lower pH, greater turbidity, and lower dissolved oxygen (DO) concentrations than lagoons that were not accessible by wild pigs, but nutrients and aquatic communities did not differ between treatments. Kaller and Kelso (2003) examined water quality in a single watershed in Louisiana and reported greater fecal coliforms (FC) and the presence of pathogenic bacteria at locations with evidence of wild pig activity. In contrast to Doupé et al. (2010), they did not observe differences in DO and stream habitat due to wild pig disturbance. Singer et al. (1984) observed greater NO_3^- concentrations in stream water from a rooted hardwood stand in Great Smoky Mountains National Park in Tennessee compared with stream water from an unrooted stand but did not find a difference in suspended solids. Two studies conducted in tropical forests in Hawaii analyzed runoff from fenced and unfenced plots with wild pig activity, and neither found differences in concentration of suspended solids or fecal bacteria due to fencing treatment (Dunkell, Bruland, Evensen, & Litton, 2011; Dunkell, Bruland, Evensen, &

Walker, 2011; Strauch et al., 2016). Brooks et al. (2020) compared runoff from a paddock containing wild pigs with a nearby stream, but runoff samples did not significantly differ from stream samples in concentrations of NO_3^- and NO_2^- , NH_4^+ , fecal bacteria, or pathogenic bacteria. Wild pigs have been found to carry waterborne pathogens such as *Giardia* and *Cryptosporidium* (Atwill et al., 1997; Hampton, Spencer, Elliot, & Thompson, 2006), but, to our knowledge, no study to date has been able to link waterborne pathogens in the environment to the presence of wild pigs.

The substantial variability in reported results, experimental designs, environmental conditions, and land cover and use of previous studies examining the impacts of wild pigs on water quality has created considerable confusion. As a result, our goal was to examine the impacts of wild pigs on water quality in headwater riparian systems using an experimental design that used the conditions we felt must be met for a thorough assessment of wild pig impacts on water quality. These conditions include: (a) Wild pigs must be present in and have access to the area being studied, (b) the pigs must be free to exhibit natural behavior, (c) the receiving body of water should represent natural flow, and (d) the water sampling technique must be sufficiently rigorous to detect subtle changes against a backdrop of high variability. Studies that use these guidelines would have greater capability of assessing the effects of wild pigs on water quality and can subsequently be used for guiding wild pig management and control initiatives to safeguard water quality in local watersheds. Our specific research objectives were (a) to identify multiple small watersheds with free-roaming wild pigs to observe changes in water quality parameters in a natural setting with minimal background variability and human activity, (b) to determine impacts on water quality by measuring nutrient concentrations and physiochemical parameters in forested headwater stream systems, and (c) to determine impacts on fecal bacteria concentrations by analyzing stream water for the presence of swine fecal bacteria and quantifying *E. coli* and fecal coliform concentrations.

2 | MATERIALS AND METHODS

2.1 | Study area

We worked at a privately owned property with a high density of wild pigs (i.e., treatment area) and on the Tuskegee National Forest (4,554 ha), which had very low pig activity and served as our reference area. The treatment area was a 4,515-ha property located in Bullock County, AL. Wildlife management practices focused on maintaining healthy white-tailed deer and eastern wild turkey (*Meleagris gallopavo silvestris*) populations. The most common habitat types were mixed pine (*Pinus* spp.)–hardwood forest and ripar-

ian hardwoods. The canopy was primarily composed of sweetgum (*Liquidambar styraciflua* L.), loblolly pine (*Pinus taeda* L.), and southern shagbark hickory [*Carya carolinacaeptentrionalis* (Ashe) Engl. & Graebn.], and the understory was mainly herbaceous and semi-woody species, such as blackberry (*Rubus* spp.), American beautyberry (*Callicarpa americana* L.), and eastern baccharis (*Baccharis halimifolia* L.). Wild pigs were present throughout the property, and camera surveys (conducted as part of a larger study) estimated the density to be 15.5 pigs km^{-2} , which is much greater than the average density of 6–8 pigs km^{-2} in the southeastern United States (Lewis et al., 2019). The reference area was located approximately 25 km from the treatment area. The areas were similar in terms of stream gradient, forest cover and habitat type, and stream size. Although wild pigs were present in some areas of Tuskegee National Forest, they were not established in the area selected for the study. We confirmed this with camera surveys conducted in March 2018.

Sampling sites (watersheds) at the treatment and reference areas were selected if they met the following criteria: low gradient, occupied by deciduous wetland forests, and streams third order or lower in magnitude. Both study areas were located in the Upper Coastal Plain physiographic region and in the Mantachie-Iuka-Bibb soil association. We selected 11 watersheds at the treatment area and three watersheds at the reference area as sampling sites. The main tributaries were perennial, whereas most of the lower-order streams were intermittent, with flow only in winter and spring. At the treatment area, damage as a result of pig activity (rooting, digging, and wallowing) was observed at all sampling sites. This activity was observed on the floodplains and within the stream channels, even when the channels were dry.

2.2 | Collection and analysis of water samples

Sampling began in May 2018 at the treatment sites and in December 2018 at the reference sites and continued through June 2019. Water samples were collected from each site ($n = 14$) every 2 wk throughout the year as long as flow was present. Sampling events at the treatment and reference areas occurred within 24 h of each other. At each site, a 500-ml grab sample was collected in the middle of the channel at the outlet point where the main stream of the watershed flowed into the connecting tributary, thereby capturing the cumulative effect of wild pigs within the small watersheds. Discharge at the sampling location was measured using the USGS mechanical current-meter method (Turnipseed & Sauer, 2010), in addition to DO, specific conductivity, temperature, and pH.

Water samples were kept on ice and transported to the Auburn University Biogeochemistry Laboratory where they were stored at 4 °C. Ion chromatography (Dionex ICS-1500,

Thermo Fisher Scientific) was used to measure concentrations (mg L^{-1}) of anions (NO_2^- , NO_3^- , SO_4^{2-} , PO_4^{3-}) and cations (NH_4^+ , K^+ , Mg^{2+} , Ca^{2+}). Dissolved organic C (DOC) and total N (TN) concentrations (mg L^{-1}) were measured using high-temperature combustion techniques (Shimadzu TOC-VCPN, Shimadzu Scientific Instruments). Concentrations of total suspended solids (TSS) were determined using filtration methods in accordance with USEPA guidelines (USEPA, 1999). Discharge and nutrient concentrations were multiplied to obtain instantaneous loads in milligrams per second (mg s^{-1}).

2.3 | Analysis of fecal bacteria

Water samples for *E. coli* analysis were taken from the grab samples immediately after collection. Three 1-ml subsamples were pipetted from each grab sample into vials containing Coliscan Easygel (Micrology Laboratories). The contents of each vial were transferred to petri dishes ($n = 3$) and incubated at 29–37 °C for 30 h. After incubation, colony-forming unit (cfu) counts were conducted using a microscope to aid in the identification of colony type by medium color (purple/blue for *E. coli* and pink/red for FC). The mean cfu for each water sample was calculated and multiplied by 100 ml to obtain the concentrations of *E. coli* and FC, in units of $\text{cfu } 100 \text{ ml}^{-1}$. Fecal bacteria concentrations were multiplied by discharge to obtain instantaneous loads in cfu per second.

Microbial source tracking (MST) techniques using swine fecal bacteroidetes were used to determine if feces from wild pigs were entering the watersheds (Okabe et al., 2007). Additional water samples (one per site and collected using the method previously described) were collected during five sampling events: June 2018, July 2018, December 2018, April 2019, and August 2019. The samples were sent to a private laboratory (Source Molecular) to test for the presence of swine fecal bacteroidetes using quantitative polymerase chain reaction. Upon arrival, each water sample was filtered through a 0.45- μm membrane filter, which was then placed in a 2-ml tube containing beads and a lysis buffer. The sample was homogenized for 1 min and DNA extracted using a DNA-EZ ST1 extraction kit (GeneRite). Amplifications to detect the target gene biomarker were run on a StepOnePlus real-time thermal cycler (Applied Biosystems) in a final reaction volume of 20 μl sample extract, forward primer, reverse primer, probe, and an optimized buffer. All assays were run in duplicate, and quantification was achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers. A positive and negative control were run alongside the samples to identify any false negatives or positives (Source Molecular, personal communication, 2019).

2.4 | Statistical analysis

Statistical analyses were conducted using R statistical platform version 3.5.3 (R Core Team, 2019). Homoscedasticity and normality of the residuals were assessed visually with diagnostic graphs and statistically using Shapiro–Wilk and Levene’s tests. Data that did not meet assumptions of normality were natural log transformed.

Linear mixed effects analysis was used to account for temporal autocorrelation and for the confounding relationship between presence of wild pigs (treatment type) and study area. Models were developed using the lme4 (Bates, Mächler, Bolker, & Walker, 2015) and lmerTest (Kuznetsova et al., 2017) packages to assess the importance of treatment type, season, and discharge in explaining water quality and fecal bacteria at the two study areas. For concentrations, a complete model was built with the fixed effects of Treatment Type (wild pigs or reference), Season (wet or dry), and Discharge, as well as interaction terms for Treatment Type \times Season and Season \times Discharge. Seasons were delineated by flow, which was greatest from November through April (wet season) and diminished from May through October (dry season). Discharge was included as a fixed effect to account for changes in nutrient and fecal bacteria concentrations due to fluctuations in stream flow. A random effect of Stream was included to account for inherent differences among watersheds and sampling areas. Additionally, a random effect of Time (day nested within month) was added to account for temporal autocorrelation as a result of repeated sampling (Chaves, 2010). The complete model for instantaneous loads included Treatment Type, Season, an interaction term for Treatment Type \times Season, and the random effects of Stream and Time.

A step-down model-building approach was used via the step function in the lmerTest package to eliminate nonsignificant ($\alpha > .05$) fixed effects and interaction terms, resulting in a final model for each water quality and fecal bacteria variable. Nonsignificant fixed effects were left in the model if interaction terms containing the effects were significant. Akaike’s Information Criterion corrected for small sample size was used to further evaluate models and confirm that the final model best fit the data.

3 | RESULTS

Fourteen streams were sampled from May 2018 to June 2019 during 16 sampling events that occurred during flow periods at bimonthly intervals. Each stream was sampled at least four times. Flow ceased at the end of July 2018 due to low rainfall, and flowing water did not return until December 2018. A drought from mid-May 2019 to mid-October 2019 meant that the majority of streams were dry or only contained

stagnant pools of water and therefore were unable to be sampled.

3.1 | Model selection

The fixed effect of treatment type was significant in linear mixed effects models for specific conductivity and concentrations of SO_4^{2-} , Ca^{2+} , TN, and DOC (Table 1). An interaction term for Treatment Type \times Season was significant in models for Mg^{2+} and NO_2^- concentrations. None of the fixed effects or interaction terms was significant for pH or PO_4^{3-} and therefore were excluded from the models. The effect of Treatment Type was significant in the model for *E. coli* but not significant for FC. An interaction term for Treatment Type \times Season was included in the FC model. For instantaneous loads, Treatment Type was significant for NO_3^- , TN, DOC, and FC models. An interaction term for Treatment Type \times Season was significant for NO_2^- , TN, DOC, *E. coli*, and FC instantaneous loads.

3.2 | Water quality

Discharge was generally greater at reference watersheds than at treatment watersheds (Table 2), although stormflow conditions resulted in considerable variation in discharge rates. The concentration of DOC in treatment watersheds was 10.62 mg L^{-1} greater than in reference watersheds ($p < .001$), with median concentrations of 17.83 and 4.51 mg L^{-1} , respectively. Treatment watersheds had 2.35 times the TN concentration of reference watersheds ($p = .01$). Concentrations of NO_2^- , NO_3^- , and NH_4^+ did not significantly differ between treatment types ($p > .05$), although NO_3^- concentration in the dry season was 0.1 times the concentration in the wet season ($p = .004$).

Specific conductivity was affected by treatment type and discharge. At treatment watersheds, specific conductivity was 3.35 times the specific conductance at reference watersheds ($p < .001$). Concentrations of SO_4^{2-} were affected by treatment type, season, and discharge, whereas Ca^{2+} was affected by treatment type and discharge. Treatment watersheds had 10.25 times the SO_4^{2-} concentration in reference watersheds ($p < .001$) and 4.84 times the Ca^{2+} concentration ($p < .001$). Median PO_4^{3-} concentrations were 0 mg L^{-1} at both treatment and reference watersheds and did not differ by treatment type, season, or discharge. Dissolved oxygen differed by season and discharge but not by treatment type. For every 1% increase in discharge, DO increased by $4.74 \times 10^{-3} \text{ mg L}^{-1}$ ($p < .001$). Concentration of TSS was affected by discharge but not by treatment type or season. Reference watersheds had greater instantaneous loads of NO_3^- ($p = .03$), TN ($p = .01$), and DOC ($p = .04$) than treatment watersheds.

3.3 | Fecal bacteria

Treatment watersheds had 40.4 times the *E. coli* concentrations of reference watersheds ($p < .001$). Concentrations ranged from 0 to 70,767.00 cfu 100 ml^{-1} at treatment watersheds and from 0 to 967.00 cfu 100 ml^{-1} at reference watersheds. *Escherichia coli* concentrations varied by treatment type and individual watershed (Figure 1). Fecal coliform concentrations ranged from 1,133.00 to 388,767.00 cfu 100 ml^{-1} at treatment watersheds and from 500.00 to 27,433.00 cfu 100 ml^{-1} at reference watersheds and were not statistically different between treatments ($p = .15$; Figure 2).

Instantaneous loads of *E. coli* ranged from 0 to 19,775,561.10 cfu s^{-1} at treatment watersheds and from 0 to 18,436.03 cfu s^{-1} at reference watersheds. Differences in *E. coli* loads between treatment types were not significant ($p = .73$). At treatment watersheds, instantaneous loads of FC ranged from 39.71 to 30,941,723.00 cfu s^{-1} , whereas loads at reference watersheds ranged from 3162.39 to 3,686,263.00 cfu s^{-1} . Instantaneous loads at reference watersheds were 80.92 times the loads at treatment watersheds ($p < .001$).

A total of 38 samples from the 14 watersheds were sent for DNA analysis of swine fecal biomarkers from five different sampling events (Table 3). Overall, DNA from swine fecal bacteroidetes was detected in 23 of 33 (69.7%) samples from treatment watersheds and in 0 of 5 (0.0%) samples from reference watersheds. Biomarker concentrations were quantified in 16 of 23 (69.6%) samples, whereas the remaining seven samples had concentrations below the limit of quantification. Quantified concentrations ranged from 361 to 19,200 copies 100 ml^{-1} , with an overall mean of 4,070 copies 100 ml^{-1} . Although some samples from treatment watersheds tested negative, each of the 11 treatment watersheds tested positive for swine fecal bacteroidetes at least once during the course of the study.

4 | DISCUSSION

4.1 | Water quality

Concentrations of DOC and TN (organic and inorganic) were elevated at the treatment watershed compared with the reference watershed; however, concentrations of NO_2^- , NO_3^- , and NH_4^+ were not elevated. Organic N content was likely elevated, which in turn increased TN concentrations. Inorganic N levels may not have been elevated due to low DO levels in the dry season. The transformation of NH_4^+ to NO_2^- is the rate-limiting step of nitrification and is slowed when oxygen is not readily available. Wild pig feces and urine likely contributed to the increased levels of organic C and N in the treatment watersheds because reference watersheds were similar in geomorphology, hydrology, and habitat type. Singer

TABLE 1 Linear mixed effects models for concentrations (top) and instantaneous loads (bottom) of water quality parameters at treatment and reference watersheds from May 2018 through June 2019

Variable	Treatment type			Season			Discharge			Treatment type × season			Season × discharge		
	Est.	95% CI	P value	Est.	95% CI	P value	Est.	95% CI	P value	Est.	95% CI	P value	Est.	95% CI	P value
NO ₂ ⁻ , mg L ⁻¹	0.08	8.40 × 10 ⁻⁴ to 0.16	.05	0.12	0.04–0.21	.004				0.20	0.14–0.25	<.001			
DOC, mg L ⁻¹	10.62	6.29–14.95	<.001				-3.97 × 10 ⁻³	-7.30 × 10 ⁻³ to -6.41 × 10 ⁻⁴	.02						
Ca ²⁺ , mg L ⁻¹	4.84	2.50–8.76	<.001				-0.07	-0.09 to -0.04	<.001						
NH ₄ ⁺ , mg L ⁻¹							-0.02	-0.03 to -0.01	<.001						
NO ₃ ⁻ , mg L ⁻¹				0.10	0.04–0.16	.004	-5.65 × 10 ⁻⁴	-1,768.99–0.01	.93				0.2	2.64 × 10 ⁻³ to 0.04	.02
K ⁺ , mg L ⁻¹							-0.04	-0.06 to -0.01	.002						
Mg ²⁺ , mg L ⁻¹	1.33	0.86–2.06	.20	1.04	0.86–1.26	.65	-0.07	-0.09 to -0.05	<.001	1.31	1.05–1.64	.02			
TN, mg L ⁻¹	2.35	1.38–4.00	.01												
SO ₄ ²⁻ , mg L ⁻¹	10.25	5.67–18.50	<.001	1.29	1.04–1.61	.03	-0.11	-0.16 to -0.06	<.001	1.12	1.04–1.20	.003			
DO, mg L ⁻¹				2.57	1.55–3.59	<.001	4.74 × 10 ⁻³	2.68 × 10 ⁻³ to 6.69 × 10 ⁻³	<.001	-4.15 × 10 ⁻³	-6.70 × 10 ⁻³ to -1.60 × 10 ⁻³	.002			
TSS, mg L ⁻¹							-0.11	-0.21 to -0.01	.03						
Sp Cond, μS cm ⁻¹	3.35	2.10–5.33	<.001				-0.11	-0.17 to -0.06	<.001						
Temperature, °C				-8.72	-10.76 to -6.69	<.001									

(Continues)

TABLE 1 (Continued)

Variable	Treatment type			Season			Discharge			Treatment type × season			Season × discharge		
	Est.	95% CI	P value	Est.	95% CI	P value	Est.	95% CI	P value	Est.	95% CI	P value	Est.	95% CI	P value
<i>E. coli</i> , cfu 100 ml ⁻¹	40.40	10.06–153.95	<.001												
FC, cfu 100 ml ⁻¹	1.13	–0.25–5.09	0.15	4.76	1.06–15.12	.002	0.09	0.02–0.18	.02	8.14	1.59–31.20	<.001			
SO ₄ ²⁻ , mg s ⁻¹				5.97	1.89–18.89	.005									
NO ₂ ⁻ , mg s ⁻¹	4.09	0.30–54.76	.28	30.42	2.17–427.09	.01	58.67	2.61–1.32 × 10 ³	.01						
NO ₃ ⁻ , mg s ⁻¹	9.32	1.37–63.36	.03												
PO ₄ ³⁻ , mg s ⁻¹				5.39	1.30–22.41	.03									
Ca ²⁺ , mg s ⁻¹				3.91	1.15–13.28	.04									
Mg ²⁺ , mg s ⁻¹				5.77	1.75–19.04	.008									
K ⁺ , mg s ⁻¹				5.45	1.51–19.64	.01									
NH ₄ ⁺ , mg s ⁻¹				2.92	1.13–7.50	.03									
TN, mg s ⁻¹	17.04	1.85–157.38	.01	1.34	0.21–8.51	.76	14.19	1.57–128.08	.02						
DOC, mg s ⁻¹	11.10	1.15–107.22	.04	1.13	0.20–6.53	.89	12.05	1.50–97.03	.02						
<i>E. coli</i> , cfu s ⁻¹	2.09	0.02–156.34	0.73	267.83	4.38–1.64 × 10 ⁴	.01	2,323.89	17.76–3.0 × 10 ⁵	.003						
FC, cfu s ⁻¹	80.92	9.03–724.95	<.001	5.63	0.79–40.16	.09	146.73	13.99–1.54 × 10 ³	<.001						

Note. cfu, colony-forming units; CI, confidence interval; DO, dissolved oxygen; DOC, dissolved organic C; FC, Fecal coliforms; Sp Cond, specific conductivity; TN, total N; TSS, total suspended solids. Estimates and confidence intervals have been back-transformed from log scale.

TABLE 2 Summary of water quality concentrations and instantaneous loads for treatment and reference watersheds from May 2018 through June 2019

Variable	Treatment		Reference	
	Median	SE	Median	SE
Concentrations				
Discharge, L s ⁻¹	1.29	1.72	5.40	2.56
SO ₄ ²⁻ , mg L ⁻¹	14.13	1.41	1.56	0.14
NO ₂ ⁻ , mg L ⁻¹	0.04	0.01	0.04	0.03
NO ₃ ⁻ , mg L ⁻¹	0.04	0.01	0.03	0.11
PO ₄ ³⁻ , mg L ⁻¹	0	3.98 × 10 ⁻³	0	8.14 × 10 ⁻³
Ca ²⁺ , mg L ⁻¹	16.76	0.59	0.83	0.38
Mg ²⁺ , mg L ⁻¹	1.50	0.05	1.13	0.20
K ⁺ , mg L ⁻¹	1.42	0.04	1.12	0.09
NH ₄ ⁺ , mg L ⁻¹	0	0.01	0	0.01
TN, mg L ⁻¹	0.40	0.02	0.12	0.02
DOC, mg L ⁻¹	17.83	0.40	4.51	0.40
TSS, mg L ⁻¹	0.03	0.02	0.01	0.01
Sp Cond, μS cm ⁻¹	0.13	0.01	0.02	0.01
pH	6.76	0.05	6.15	0.12
<i>Escherichia coli</i> , cfu 100 ml ⁻¹	333.00	632.91	0	30.16
FC, cfu 100 ml ⁻¹	8,067.00	3,738.25	3,000.00	1,156.64
Instantaneous loads				
SO ₄ ²⁻ , mg s ⁻¹	15.9	16.5	5.4	4.8
NO ₂ ⁻ , mg s ⁻¹	0	0.2	0.1	1.0
NO ₃ ⁻ , mg s ⁻¹	1.4 × 10 ⁻²	0.1	0.2	0.6
PO ₄ ³⁻ , mg s ⁻¹	0	1.9e ⁻²	0	0.1
Ca ²⁺ , mg s ⁻¹	17.6	15.1	6.7	2.2
Mg ²⁺ , mg s ⁻¹	1.8	1.8	5.8	2.7
K ⁺ , mg s ⁻¹	1.6	2.6	6.7	2.4
NH ₄ ⁺ , mg s ⁻¹	0	4.3 × 10 ⁻²	0	3.2 × 10 ⁻²
TN, mg s ⁻¹	0.4	0.9	0.6	0.3
DOC, mg s ⁻¹	19.9	29.5	21.4	12.5
TSS, mg s ⁻¹	3.0 × 10 ⁻²	0.2	0.1	0.1
<i>E. coli</i> , cfu s ⁻¹	3522.3	16.3 × 10 ⁵	0	819.9
Fecal coliforms, cfu s ⁻¹	9.4 × 10 ⁴	3.2 × 10 ⁵	2.1 × 10 ⁵	1.3 × 10 ⁵

Note. DOC, dissolved organic C; FC, fecal coliforms; Sp Cond, specific conductivity; TN, total N; TSS, total suspended solids.

et al. (1984) found numerically greater annual N concentrations in stream water from an area with rooting activity; however, statistical comparison between treatment types in that study was not performed because drought conditions reduced the number of samples collected. Increased precipitation and stormflow during the wet season could have transported nutrients and organic material from the floodplain into the watershed, resulting in elevated N concentrations in stream water. The geomorphology and hydrology of the two sampling locations was not described in the Singer et al. (1984) study, but differences in slope, substrate, basin shape, watershed size, and stream flow could have contributed to different N concentrations in stream water. In comparison, Brooks et al. (2020)

did not find a link between runoff from wild pigs and N in stream water. However, the animals in that study were in a pen and did not have access to the stream, and riparian vegetation acted as a buffer for pen runoff. Other factors present in the study area likely influenced N and other nutrient levels in the stream, including the presence of livestock, agriculture, and construction.

Dissolved oxygen did not significantly differ between treatment types but differed by season and stream discharge. Across watersheds and treatments, DO was lower in the dry season and positively correlated with discharge. Dissolved oxygen content is affected by several factors, including water temperature, flow, photosynthesis, and microbial

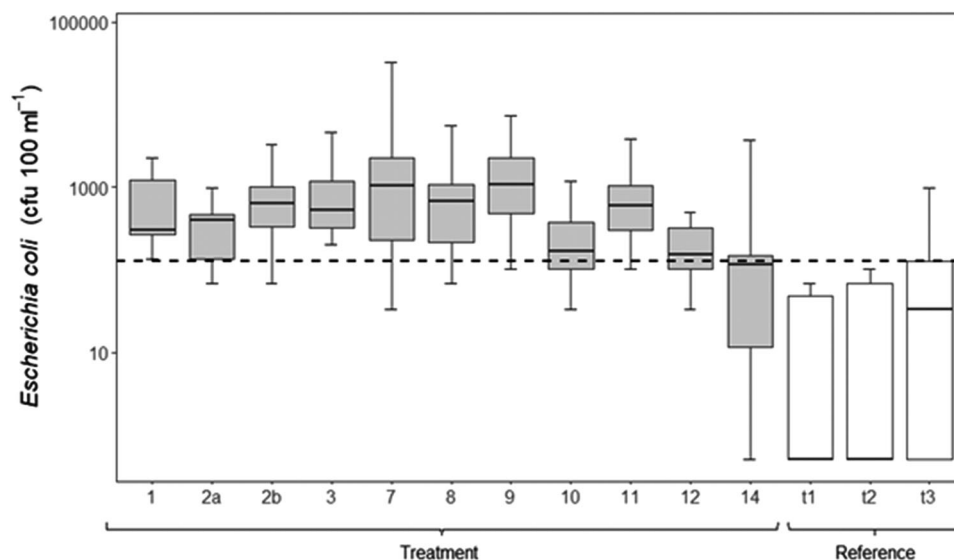


FIGURE 1 *Escherichia coli* concentrations for treatment and reference watersheds from May 2018 through June 2019. The dashed line indicates the USEPA's recommended maximum geometric mean for *E. coli* concentrations in recreational watersheds (126 cfu 100 ml⁻¹). The y axis is on a log scale

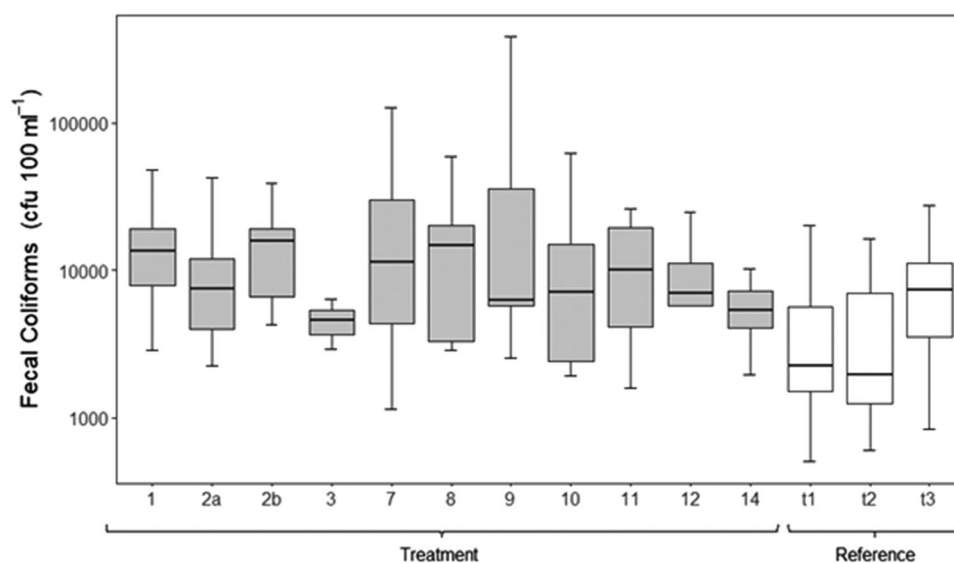


FIGURE 2 Fecal coliform concentrations for treatment and reference watersheds from May 2018 through June 2019. The y axis is on a log scale

decomposition of organic material. Although disturbance (i.e., rooting) and introduction of animal waste in the aquatic-terrestrial interface can increase organic material in a stream and thereby increase microbial consumption of DO, we did not find significantly lower levels of DO in watersheds with wild pigs. We speculate that the watersheds included in this study had little to no riparian vegetation buffer between the floodplain and the stream channel, so there was very little plant matter that could be transported into the aquatic environment by rooting activity. We attribute the elevated DOC and TN in the treatment watersheds to wild pig feces, which suggests that microbial consumption of DO increased in order to decompose the increased amount of organic matter. The fact that we did not detect a decrease in DO levels could

be due to spatial and temporal variability in the watersheds and wild pig usage of these habitats, especially if the change was subtle. A larger sample size and more intensive sampling may be needed to observe changes in DO levels resulting from wild pig activity. Doupe et al. (2010) reported lower DO in ephemeral lagoons accessible to wild pigs; however, the lagoons significantly differed in plant, macroinvertebrate, and fish species composition, all of which are factors that can affect DO levels. Additionally, they sampled during the dry season, which is when water levels, and subsequently DO, continuously decrease because there is no replenishment from rainfall or runoff.

Specific conductivity, Ca²⁺, and SO₄²⁻ were greater in the treatment watersheds but differed by season and/or

TABLE 3 Results from DNA analysis of swine fecal biomarkers in water samples collected at treatment and reference watersheds during 2018–2019

Treatment watershed	June 2018	July 2018	December 2018	April 2019	August 2019
	copies 100 ml ⁻¹				
1	ND	DNQ	569	–	–
2A	DNQ	– ^a	18,300	692	–
2B	ND	–	19,200	ND	–
3	DNQ	ND	826	–	–
7	ND	–	666	–	–
8	361	ND	5,210	ND	–
9	3,540	–	1,290	–	–
10	ND	–	1,900	DNQ	–
11	577	–	10,500	622	–
12	ND	ND	619	DNQ	–
14	DNQ	–	1,050	DNQ	–
Reference					
T1	–	–	–	ND	ND
T2	–	–	–	ND	–
T3	–	–	–	ND	ND
Detections/total	6/11	1/4	11/11	5/7; 0/3	0/2

Note. DNQ, detected not quantified (concentration below limit of quantification); ND, not detected.

^aSamples were not analyzed from this watershed.

discharge. Sulfur is released during the decomposition of organic material and oxidized to SO_4^{2-} , so wild pig feces could have increased SO_4^{2-} in the treatment watersheds. However, conductivity, Ca^{2+} , and SO_4^{2-} are strongly influenced by soil type and subsurface geology, and differences in concentrations are most likely due to geologic and soil morphologic variability. Greater Ca^{2+} concentrations at the treatment watersheds may be attributed to subsurface marine deposits referred to as the Selma Chalk region, which does not run under the reference watersheds. A possible reason for the difference in SO_4^{2-} concentrations between the treatment and reference watersheds is the original water source. The treatment watersheds may be fed more by underground springs or may not be in comparison to the reference watersheds. Groundwater erodes and dissolves rock and minerals over time, which introduces SO_4^{2-} ions to the aquifer (USEPA, 2003). Conductivity reflects the concentration of ions present (USEPA, 2012b), so elevated specific conductivity at treatment watersheds is a function of greater Ca^{2+} and SO_4^{2-} concentrations.

Although wild pig rooting was regularly observed within and adjacent to the stream channel, there was no difference between TSS concentrations in treatment and reference watersheds. The soils at both locations were mainly composed of sandy bedloads, which means sediment particles drop quickly out of the water column and are not suspended for long. This is likely why PO_4^{3-} was not elevated despite

the input of fecal material and urine from wild pigs, as it binds quickly to sediment particles (Søndergaard, Jensen, & Jeppesen, 2003). Also, there was little to no overland runoff crossing the terrestrial–aquatic interface because the floodplains at all watersheds were relatively flat. Although we did not detect a difference in TSS between treatments in this study, other watersheds with different geomorphological and hydrological features (i.e., V-shaped catchment with clay substrate) could show more pronounced differences. Dunkell, Bruland, Evensen, & Litton (2011) and Strauch et al. (2016) did not find a significant difference in TSS in runoff from fenced and unfenced plots with wild pig presence. However, plot size was small (10 m by 5 m) in comparison to the amount of soil that wild pigs can disturb, and unfenced plots may not have experienced the intensity of rooting typical for the area. Water samples were only taken from stormflow runoff, which can highlight extreme values and does not reflect water quality under normal conditions.

Unlike nutrient concentrations, instantaneous loads for TN, NO_3^- , and DOC were greater at reference watersheds than at treatment watersheds. However, reference watersheds typically had greater discharge, especially during times of increased precipitation and decreased evapotranspiration, and therefore nutrient loads were greater despite low concentrations. Additionally, much of the load data were highly variable due to differences in discharge, which made it difficult to detect differences between treatment types.

4.2 | Fecal bacteria

There were stark differences in *E. coli* concentrations between the two watershed treatment types. The USEPA recommends that recreational watersheds have a maximum geometric mean concentration of 126 cfu 100 ml⁻¹ in a 30-d sampling period and that no more than 10% of samples taken have a concentration greater than the statistical threshold value of 410 cfu 100 ml⁻¹ (USEPA, 2012a). All treatment watersheds had mean *E. coli* concentrations that exceeded 126 cfu 100 ml⁻¹, whereas the reference watersheds were below this threshold. Median *E. coli* concentrations at the treatment watersheds were similar to those measured in nearby urban watersheds (Crim, Schoonover, & Lockaby, 2012), despite the lack of surface runoff from developed areas and other anthropogenic sources at our study sites. Treatment and reference watersheds both lacked livestock and had the same wildlife species (and sources of fecal matter) except for wild pigs; therefore, the elevated *E. coli* concentrations at the treatment watersheds were likely a result of wild pig activity.

Unlike *E. coli*, FC concentrations did not vary by treatment type and were elevated in comparison to nearby urban watersheds (Crim, Schoonover, & Lockaby, 2012). Although instantaneous loads of FC were greater at reference watersheds, this can be attributed to greater discharge rates, as previously mentioned. Fecal coliform concentrations historically were used to predict the presence of gastrointestinal illness-causing pathogens, but the USEPA no longer uses FC as an indicator of fecal contamination (USEPA, 2012a). *Escherichia coli* and enterococci are now the preferred method of identifying bodies of water potentially contaminated by fecal material because their presence unequivocally indicates the occurrence of fecal contamination even if the detected strains are nonpathogenic (Edberg, Rice, Karlin, & Allen, 2000). Fecal coliform testing also detects thermotolerant nonfecal ("environmental") coliform bacteria and can lead to an overestimation of fecal contamination and risk to public health (Francy, Myers, & Metzker, 1993). In our study, it is likely that environmental coliform bacteria were naturally present in runoff and streams and incubated along with FC, which made it difficult to detect an effect of wild pig presence on FC concentrations. Previous studies examining the impacts of wild pigs on fecal contamination of watersheds have reported mixed results. Kaller and Kelso (2003) reported positive correlation of wild pig presence with fecal bacteria, but they measured FC, and sampling occurred only three times in one watershed basin. Dunkell, Bruland, Evensen, and Walker (2011) and Strauch et al. (2016) did not find a significant effect of wild pigs on *E. coli*, enterococci, or total coliforms in runoff from fenced and unfenced plots, but, as mentioned previously, plot sizes were small, and unfenced plots may not have experienced typical rooting intensity. Brooks et al. (2020) did not detect a difference in *E. coli* and ente-

rococci concentrations between pen runoff and nearby stream water, which is likely because wild pigs did not have direct access to the stream and because there were other sources of fecal matter in the area, such as livestock and human waste.

The positive detection of swine fecal bacteroidetes in treatment watersheds via MST and the absence of same in reference watersheds further indicate that wild pigs can introduce fecal material and disease-causing pathogens to streams. The effects of fecal contamination are not limited to the initial source area: downstream areas may be affected as well. Water-borne bacteria and pathogens accumulate as low-order streams flow into main tributaries of increasing magnitude. Stream sediments also serve as a reservoir for *E. coli* and potentially other pathogens (Garzio-Hadzick et al., 2010), and disturbance events that affect stream sediment (e.g., stormflow, anthropogenic activities, wild pig rooting) could resuspend these microorganisms in the water column and cause them to travel farther downstream or come into contact with a susceptible human or animal. Reducing wild pig presence in riparian areas during times of potential disturbance could reduce the amount of fecal contamination in the watershed and thereby decrease the *E. coli* deposited in stream sediment. To our knowledge, this study is the first that definitively links wild pig presence to the introduction of fecal material and waterborne pathogens in watersheds and meets the four conditions previously mentioned that are needed for an accurate assessment of wild pig impacts on water quality.

5 | CONCLUSION

This study shows that wild pigs are a threat to water quality in forested watersheds by introducing fecal material and potentially disease-causing organisms. Further research on the downstream fate of pathogens and potential sources of contact with humans and animals is necessary for a clear understanding of the impacts wild pigs have on local water quality and ecosystem health. Our results suggest that MST and *E. coli* monitoring may be effective ways to gauge wild pig activity in watersheds and indicate that it may be important to control wild pig populations upstream of major drinking water sources and recreational areas.

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AUTHOR CONTRIBUTIONS

Sara A. Bolds, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing-original draft, Writing-review & editing; Graeme Lockaby,

Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing-review & editing; Stephen S. Ditchkoff, Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing-review & editing; Mark D. Smith, Conceptualization, Funding acquisition, Project administration, Writing-review & editing; Kurt C. VerCauteren, Funding acquisition, Resources, Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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